Our ref: ARM-1A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: D. ARMSTRONG	:	Art Unit: 1753
Serial No. : 10/083,845	:	
Filed : February 26, 2002	:	Examiner: A. S. Noguerola
Title : METHOD FOR SEPARATION, IDENTIFICATION AND EVALUATION OF MICROBES AND CELLS	:	Dated: November 15, 2006

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION

S i r:

I, Daniel W. Armstrong, hereby declare and say as follows:

1. I am the named inventor in this case.

2. I received my B.S. degree from Washington and Lee University in 1972. I earned my M.S. degree in 1974, and my Ph.D in Chemistry in 1977 from Texas A&M University.

3. I am considered to be one of the world's leading authorities on the theory, mechanism, and use of

capillary separations. I have developed over 30 different LC and GC columns in my laboratories that have been commercialized and/or copied worldwide.

4. I am aware that the Examiner has taken the position that "…whether a separation medium is used, such as gel or neutral water-soluble polymer while separating the microbes/cells, barring a showing to the contrary, such as unexpected results, is just a matter of optimizing the separation of the microbes/cell… whether the water soluble polymer is "dilute" is just a matter of optimizing the sieving effect of the polymer".

5. To prove the unexpected nature of the use of a dilute neutral water-soluble polymer in electrophoresis separation of microbes, four tests have been run, wherein no polymer added, two tests for a dilute water soluble neutral polymer with the concentration in the range of the invention, and a water soluble polymer with concentration of 0.6%, which is below typical polymer concentrations used in the electrophoresis.

6. Each one of the tests reported herein was performed by me or under my direct supervision and control.

7. The conditions for this series of tests were in accordance with the procedure at pages 21-23 of the specification. In particular, the polymer used is polyethylene oxide (PEO), as in example 1, and the microbial is *S. cerevisiae*, as in Microbe Preparation (6)(page 21). The separations were performed on a Beckman Coulter P/ACE system. The capillary used was 30 cm in length (20 cm to detection window) with a 100 micron i.d. The run buffer was pH 8.4 (3.94 m tris/0.56 mM boric acid/0.013 m M EDTA).

8. Referring to the attached figures, Fig. A shows that without PEO, the microbial injection band travel with the EOF and does not converge or separate from anything.

9. Fig. B shows the effect of additions of dilute PEO, with the concentration of 0.0125% in the running buffer (same with the concentration specified at line 5, page 23). As can be seen in Fig. B, the microbe separates and elutes after the EOF.

10. Fig. C shows that a small increase in PEO concentration can produce a dramatic increase in the microbial elution line. The test was run with a PEO concentration of 0.025%, as specified at line 7 of page 25 off my

application. As shown in Fig. C, the elution time jumps to more than 2 hours.

11. I am aware of the polymer concentration in the cited references. Such as 0.40% methylcellulose in Jenkins, at least 2.5% of PEG400 or sorbitol in Grant (last paragraph, page 30), or 0.1-0.2% linear polymer in Johnson (lines 46-47, column 10). However, as Fig. D shows that if PEO concentration goes 0.06%, which is substantially lower than the above concentrations typically used in the references cited, there is no elution of microbes even after hours.

12. PEO concentrations higher than 0.06% can not be used to separate microbes in electrophoresis. In these cases, the injected microbes do not separate from each other, nor do the microbes converge. Rather the unseparated injection plug of microbes is carried through the detector in a state similar to how it was injected as in Figure E.

13. I am aware of the use of a gel in capillary electrophoresis, such as 7% gel in the examples 2, 3, 4 of Johnson, to separate nucleic acids. From my experience, if a gel is used in the capillary for the separation of microbes, there will be no movement of the

injected microbes. They would never come through the detector because at the concentration of gel formation, the microbes will be stopped by the gel and clog the capillary.

14. I am of the opinion that only with the dilute water soluble neutral polymers in the present invention, will the different microorganisms sort out, converges and aggregate with their own kind, at different rates. By using a neutral polymer that is so diluted where one dissolved polymer molecule does not contact another, which is not sufficiently concentrated to form an entangled polymer or molecular sieve of any type, the individual microorganisms can be separated from each other as sharp, well separated peaks. This is why the present invention requires a dilute polymer - such as 0.0125% - 0.025% in the specification.

15. It is declared by undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under section 18 US Code 1001, and that such false statements may jeopardize the

validity of this application or any patent issuing thereon.

Daniel W. Armstrong

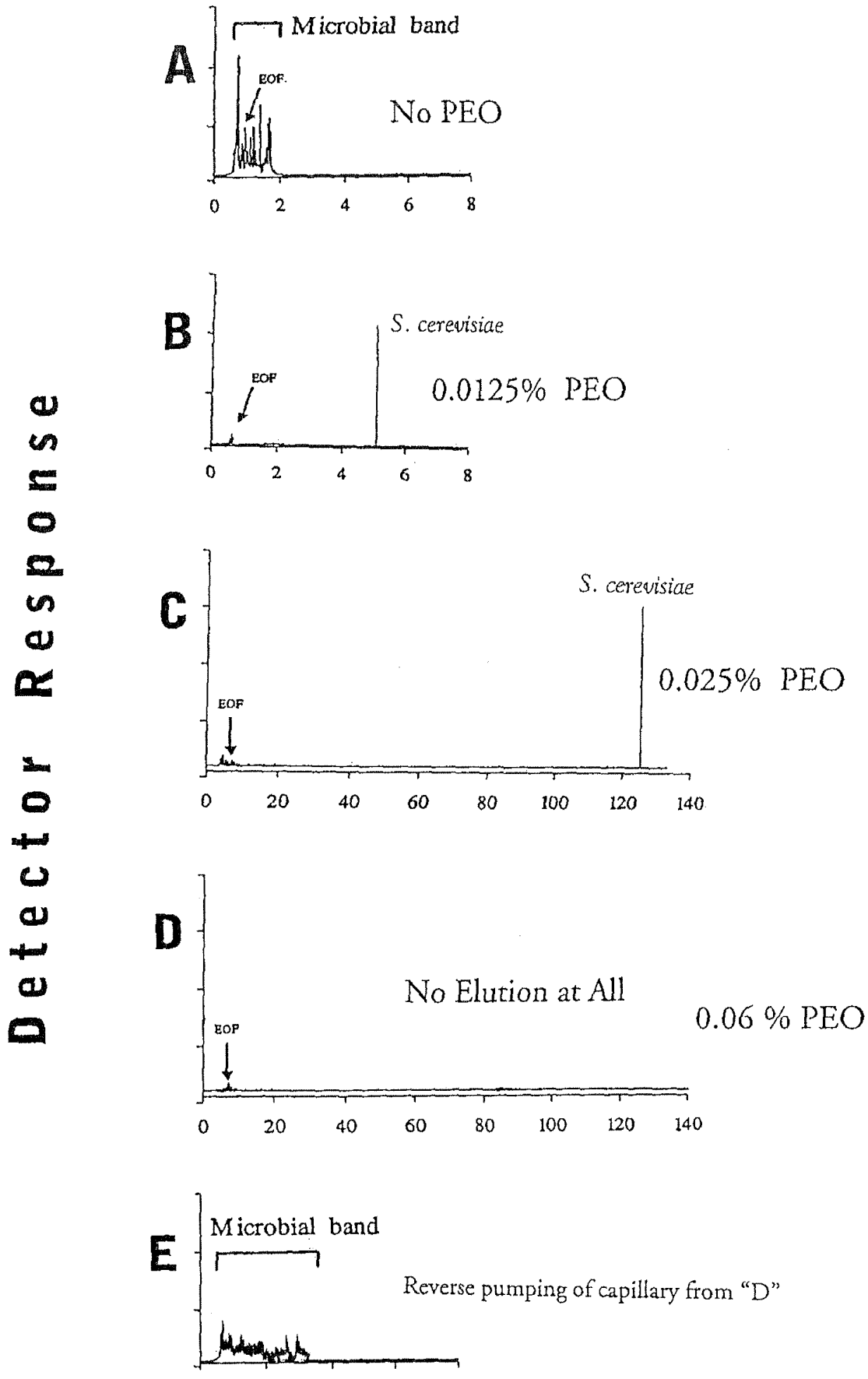
Dated: This 17th day of November, 2006

DCL/YC

Enclosures:

Figures

Two articles


Detector Response
A
Microbial band
EOF
No PEO
0
2
4
6
8
B
S. cerevisiae
EOF
0.0125% PEO
0
2
4
6
8
C
S. cerevisiae
EOF
0.025% PEO
0
20
40
60
80
100
120
140
D
No Elution at All
EOF
0.06 % PEO
0
20
40
60
80
100
120
140
E
Microbial band
Reverse pumping of capillary from "D"